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# The Reproductive Ecology of *Graptemys* *geographica* in the Central Canal

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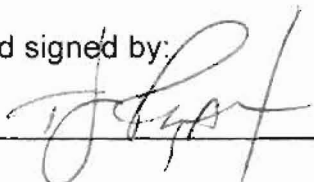
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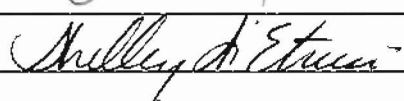
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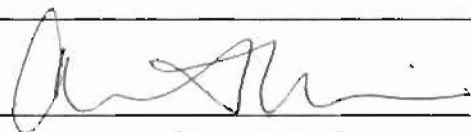
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**The Reproductive Ecology of *Graptemys geographica* in the Central Canal**

A Thesis

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and

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In Partial Fulfillment

of the Requirements for Graduation Honors

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## **PART I. LITERATURE REVIEW**

### **Life History Characteristics and Their Effect on Reproduction in Turtles**

## INTRODUCTION

Life for all organisms involves carefully managing a limited amount of resources. In many cases these resources affect how organisms live on a daily basis. In addition to competition from other species, organisms face competition within their own species, populations, and niches. Those individuals that manage their resources effectively will increase their chances of survival, reproduction, and the continuation of their genes in the gene pool.

Studies that focus on life history characteristics of organisms aim to understand the mechanisms used by organisms to increase the chances that their genes will remain in the gene pool past their own lifetime. The goal of these mechanisms is to ensure survival and maximize reproduction. The techniques used for survival are often unique for a given species or population, and studying these techniques along with their associated life history characteristics provides scientists basic information about how an organism interacts with its surrounding community. This information can then be used to understand the consequences associated with modifications (whether natural or anthropogenic) to the organism's environment, and ultimately be used to help conserve and protect organisms.

Understanding the reproductive patterns and general life history characteristics of any organism is essential in helping to manage populations throughout natural areas impacted by the encroachment of urbanization (Bager et al., 2007). Urbanization affects a variety of organisms; however, turtles have been particularly impacted by human interference and habitat loss and destruction (Klemens, 2000). Although aquatic turtles primarily need sufficient aquatic habitats, they also require terrestrial areas suitable for

nesting (Marchand and Litvaitis, 2004). The need for dual habitats makes turtles particularly susceptible to environmental modifications even when the primary aquatic habitat is regarded as protected (Burke and Gibbons, 1995). In addition to loss of habitat, invasive species, environmental pollution, disease, global climate change, and overexploitation are also contributing to the worldwide decline of the reptile population (Gibbons et al., 2000). Due to the fact that turtles have such long life expectancies, it is particularly important to conduct long-term studies in order to accurately assess their population dynamics over time (Burke et al., 1995). In light of increasing development, these long-term studies not only provide baseline data for a given population, but they are also important in determining population trends in areas where environmental modifications are occurring rapidly.

One major aspect of population dynamics is reproductive ecology. In turtles, understanding reproductive patterns involves the interaction among many different factors. Although some of these factors can be controlled at the individual or population level, others such as rainfall and temperature are beyond the scope of control of any organism (Aresco, 2004). Other non-controllable factors may include climatic variations, genetic differences and habitat availability (Aresco, 2004) within and amongst different geographic localities (Bager et al., 2007). A successful population is well-suited to live and take advantage of the surrounding biotic and abiotic factors in its particular environment despite the presence of these uncontrollable factors. Adaptations to existing conditions promote populations that are well-adapted and can survive despite competition and environmental pressures.

In order to maximize reproduction in various environments, selection pressure at the individual level facilitates the promotion of reproductive strategies that increase both maternal and offspring fitness (Hofmeyr et al., 2005). Increasing maternal and offspring fitness helps to increase the overall fitness of the population, and on a broader scale, the species. Fitness, ultimately the result of allocation of available resources, often involves balancing a series of inter-related trade-offs. Trade-offs occur when benefits associated with a change in one life history trait are associated with a cost related to another trait (van Rooij et al., 1995). For example, in females an increased level of reproductive investment will involve the trade-off of energy allocated for growth (van Rooij et al., 1995). Thus, early on in the life cycle of long-lived organisms the balance is most often shifts to growth, and after the female reaches sexual maturity the rate of growth slows, allowing more energy to be invested in reproduction (Smith and Fretwell, 1974).

Females must balance increases in their own fitness with that of their offspring. The amount of resources dedicated to offspring fitness depends on the amount of investment the mother is willing to make in order to produce viable offspring and ensure the future existence of her genetic contribution. This parental investment is defined as, “any investment by the parent in an individual offspring that increases the offspring’s chance of surviving at the cost of the parent’s ability to invest in other offspring” (Trivers, 1972). In some organisms this may be maternal care to live offspring; in turtles this investment usually involves increased yolk material (Congdon and Gibbons, 1985) as there is little parental investment once the nest is laid and the female leaves the nest while the embryos contained within grow, develop, hatch, and emerge by themselves (Wilson, 1998).

Ultimately, reproduction in turtles involves numerous facets of parental investment. Determining clutch size in a single nesting event, estimating the fecundity of an individual over its lifetime, understanding nest site placement, and calculating the survivorship of hatchling turtles and the long term viability of the population are all dependent on a series of ecological trade-offs. Reproduction in turtles involves much more than initial fertility; population survival depends on the presence of a suitable environment for offspring growth and development. Urbanization often impacts this environment and can in turn compromise the reproductive success throughout turtle populations.

## **FECUNDITY**

Fertility and ultimately fecundity are the result of the interaction between numerous different factors within a reproductive season and throughout the lifetime of a mature individual. In oviparous organisms, fecundity is measured by determining the average clutch size produced per reproductive bout and the total number of clutches produced in a lifetime. Clutch sizes vary and have been reported to range from a single egg in the world's smallest tortoise (*Homopus signatus*) (Hofmeyr et al., 2005) to 143 eggs found in a nest of the olive ridley turtle (*Lepidochelys olivacea*) (López-Castro et al., 2004). Clutch sizes in turtles are influenced by numerous factors, one of which may be body size. Internal space constraints are associated with the fact that the hard shell of the turtle prevents expansion to accommodate the eggs (Litzgus and Mousseau, 2003). Thus, space availability may be a limiting factor in determining the size of the clutch.

The clutch size of some species positively correlates to body size while in other species there is no correlation (Congdon and Gibbons, 1985). Carapace width, found to be the most statistically relevant factor, and shell height were both positively correlated to clutch size in the Eastern box turtle (*Terrapene carolina carolina*) (Wilson and Ernst, 2005). Although relationships between clutch size and body mass have been studied in depth (reviewed in Bager et al., 2007), conflicting data have been reported within and among species. Whether or not a relationship is observed may vary greatly depending on the specific environment, the previous year's growing season, the individual's age and body size, and the reproductive output of the individual in the previous year (Gibbons et al., 1979). Geographic variation may be important as well. For example, higher latitudes have been found to support higher overall reproductive rates and larger clutch sizes (Bager et al., 2007).

Clutch size varies both within and among species. Despite this variation, the overall goal is to obtain optimal hatchling fitness for the offspring produced. In order to increase the fitness of the hatchlings individual egg size should be increased before clutch size (Congdon et al., 1983). Egg size not only affects hatchling survivorship but also clutch size. Because of limited space available for egg retention, often times there is a trade-off between numerous, small-egg clutches and small clutches of greater egg size (Hofmeyr et al., 2005). This trade-off exists due to the fact that resources needed for reproduction, including energy, are limited (Harms et al., 2005). When optimal hatchling size has been reached it then becomes more beneficial to allocate more energy to increasing the number of offspring rather than offspring size.

Greater egg size does not always ensure increased survivability (Congdon et al., 1999). However, egg size does have a cascading effect as small eggs often produce smaller hatchlings who are not as fit as larger ones (Harms et al., 2005). Studies examining the relationship between egg size and clutch size provide mixed results whether or not there is a correlation between the factors (reviewed in Congdon and Gibbons, 1985). Even if larger eggs help to increase offspring fitness, the size of the eggs cannot expand indefinitely. Eggs produced can only be of a maximum width because they must be passed through the opening of the pelvic girdle (Bager et al., 2007 and Lindeman, 2005). Female body size influences the size of this aperture and thus may also limit the eggs' width, but not their length as observed in the Texas map turtle *Graptemys versa* (Lindeman, 2005).

When neither clutch size nor egg size can no longer be increased, still more energy can be used in reproductive expenditures. Greater reproductive energy allocation can result in eggs that contain a greater amount of nutrients and aid in increasing the fitness of the clutch (Harms et al., 2005). Correlation between the lipid content of the eggs and the amount of time spent overwintering has been found in some species (Congdon et al., 1983). Increasing the nutritional content of the eggs produced will not affect the physical size or number of eggs produced, yet the offspring produced will still benefit from the increase in energy allocated to reproduction.

There is not only variation within the number and size of the eggs contained in a single clutch but also within the number of clutches produced per season. Clutch frequency is one of the most important measurements of reproductive output (Gibbons et al., 1982). Clutch frequency is a measurement of the number of times an individual lays a

clutch within a season and from year to year (Tucker, 2001). Over an individual's lifetime, clutch frequency is a major contributor to the organism's fecundity. Multiple clutches produced in a season may help to dissipate the harmful effects of predation over several nests instead of a single nest (Litzgus and Mousseau, 2003). Clutch frequency may be related to the size of the female (Iverson, 1999) or may be independent of body size (Litzgus and Mousseau, 2003). Because larger females have more energy available to allocate to reproductive activities, they may exhibit patterns of multiple clutching more often than their smaller-sized turtles (Litzgus and Mosseau, 2003). Nonetheless, in South Carolina it was found that resource availability plays a greater role in determining multiple clutching than female body size (Litzgus and Mosseau, 2003).

The combination of clutch size and clutch frequency can help determine the annual reproductive potential of an individual (White and Moll, 1991). However, the amount of energy dedicated to reproduction is subject to change throughout the lifetime of an individual. As size changes with ontogeny, simultaneous changes in the amount of energy allocated toward reproduction may also occur (Harms et al. 2005). Despite the variability of clutch size and frequency, understanding how and why these variations occur is useful in determining whether or not anthropogenic modifications to the environment or other factors are responsible for changes in reproductive output. Additionally, the total reproductive output of a population can be extrapolated and population models can be designed if the clutch size, clutch frequency, and number of mature females within a population can be estimated (Bager et al., 2007). Population models aid in predicting population trends and when appropriate, constructing plans for conservation.



## NESTING

The timing of nesting and nest site selection are dependent on numerous factors. Environmental clues may trigger the onset of the nesting season. As a result, there may be significant geographic variation in the timing of nesting. For instance, nesting begins earlier in the season at lower latitudes due to warmer temperatures (Bager et al., 2007). Additionally, recent rainfall helps to soften the soil and make nest building much earlier thus increasing nest activity after periods of rain (Bager et al., 2007). If environmental cues trigger nesting when the soil is too hard to dig in, some turtles may resort to softening the soil themselves by using the water stored in their accessory bladder (Bager et al., 2007).

However, before the actual nest construction can occur, a turtle must locate a suitable site for nesting. The nest site selected by the female may depend on the individual female's nesting experience and age (Harms et al., 2005). Because the survival of her offspring often hinges on nest site selection, it is not uncommon for a female to dig several false nests before finally depositing her eggs in a nest she finds satisfactory (Wilson, 1998). The site of nest placement is crucial in determining whether or not the nest will be successful. However, the amount of suitable space near the turtle's aquatic habitat is often a limiting factor regarding nest site selection (Bager et al., 2007). Limited space may cause some turtles to nest near roadsides or in areas with limited vegetation (Bager et al., 2007). Limited space may also cause nests to be located in areas of higher densities than would otherwise be expected. Higher nest densities may increase the chance of predation and thus cause some turtles to travel further distances to nest in hopes of limiting predation of their nest (Rowe et al., 2005). Despite the increased

chances for predation, nests of northern map turtles (*Graptemys geographica*) were often located within closer proximity to one another than would be expected otherwise (Baker et al., 2003). Higher than expected nest densities may be due to limited areas of suitable for nesting or even social factors that influence turtles to nest near one another despite the availability of other suitable nest locations (Flaherty and Bider, 1984). In order to increase the chances for their offsprings' survival, older females may place their nests closer to water compared to younger females in order to increase the chances that their young hatchlings will successfully and quickly find the water's safety before becoming another organism's prey (Harms et al., 2005). This suggests that the increased amount of nesting experience older females possess, helps them to choose nesting locations that will be the most beneficial to their offspring.

Once a nest site has been selected by a female and eggs are laid, the nest becomes not only important in providing shelter and protecting the embryos from predation, but it must also serve as a location where the embryos can successfully develop (Congdon et al., 2000). Nests are critical in ensuring that the livelihood of the offspring. However, nests must remain sheltered and protected despite possible complications such as drying out, flooding, eroding away as a result of thunderstorms, or becoming encapsulated by surrounding vegetation (Congdon et al., 2000). Thus both the location of the nest and its depth become important components of a successful nest as shallow nests are more easily affected by environmental factors (Wilson, 1998). Selection pressures along with evolution have led to nest timing and placement that allows favorable conditions for embryo development and ultimately hatchling success (Buhlmann et al., 1995).

## HATCHING

When hatchling turtles emerge from their nest they leave its protection and shelter and enter into an environment where they must struggle to survive at a young age and combat environmental conditions as well as predators.

One strategy for survival used by some hatchling turtles is that of overwintering. Overwintering allows turtle embryos to develop and hatch within the protection of the nest, and it delays emergence from the nest until conditions are more favorable and the chances of survival are increased (Nagel et al., 2004). Additionally, overwintering provides hatchlings time to grow and develop in a protected environment before the young hatchlings must face the increased pressures and competition associated with living in the environment outside of their nest (Baker et al., 2003). Yet growth within the nest can only be achieved if there is access to sufficient energy reserves within the nest itself (Nagel et al., 2004). The increased amount of energy needed may be obtained from increased lipid levels found in eggs of species that overwinter (Congdon et al., 1983). Overwintering, sometimes referred to as delayed emergence, is seen in many different species, including populations of *G. geographica* throughout its range (Nagle et al., 2004).

Without overwintering, hatchlings would hatch and emerge from the nest in late fall and almost immediately prepare for hibernation in order to survive the winter (Maginnis et al., 2004). Hibernation is a successful method used by adult turtles to survive the winter; but, it is a physically demanding process that is often times too taxing for many young hatchlings that may not be fully developed nor possess sufficient energy reserves to successfully continue aerobic respiration through the winter (Maginnis et al.,

2004). Overwintering can be beneficial to young hatchling turtles as it allows them to develop in a protected environment while they are sheltered from the harsh environmental conditions occurring outside the nest; but, this survival technique is not without risks. Due to the hatchling's relatively large surface area, the chances for evaporative water loss are increased and hatchling turtles can easily become dehydrated and risk desiccation (Baker et al., 2003). In addition to avoiding desiccation, hatchlings within the nest may also have to tolerate sub-freezing temperatures as low as  $-8^{\circ}\text{C}$  (Nagle et al., 2004). The dangers associated with overwintering are offset by the hopeful increases in hatchling growth and development that gives these hatchlings an advantage over their competitors once they leave the nest.

Nest site selection becomes even more critical for species whose offspring overwinter since the newly hatched offspring live in the nest for an extended period of time. In red-eared sliders (*Trachemys scripta*), overwintering in nests located in more moist substrates reduced the amount of body mass lost over the winter and resulted in larger, more fit, hatchlings (Tucker and Paukstis, 1999). Yet when moisture levels within a nest are too high, the chance that ice will form in the nest increases and, in turn, the hatchlings become more susceptible to freezing (Baker et al., 2003). Changes in the ambient air conditions throughout the season may also change the conditions within the nest. Shallow nests dug in hard, dry soil will experience greater temperature fluctuations throughout the seasons than deeper, moister nests (Wilson, 1998). It is crucial for the female to locate areas where a relatively deep and moist nest can be dug in order to increase the chances that her young can successfully overwinter.

When conditions outside of the nest are favorable, thermal and rainfall cues aid in triggering emergence from the nest during a period of time when the hatchlings have the greatest chance of prolonged survival (Nagel et al., 2004). Once emergence begins, the nest becomes more susceptible to predation due to the olfactory and visual signals the hatchlings unintentionally provide predators (Congdon et al., 2000). Thus, synchronous nest emergence reduces hatchling mortality as asynchronous emergence increases the chances that predators will find the defenseless hatchlings (Congdon et al., 2000). After emergence from the nest, hatchling turtles must make their way towards water. However, turtles may not naturally orient towards water; instead random dispersal may occur (Congdon et al., 1999). Wood turtle hatchlings (*Glyptemys insculpta*) are thought to be guided to water by a combination of olfaction, vision, positive geotaxis, and other cues detected by their low-frequency hearing (Tuttle and Carroll, 2005). It is advantageous for hatchlings to be oriented to water as quickly as possible since increased time on land spent searching for water increases the hatchling's exposure and puts it at a greater risk for predation (Congdon et al., 1999). Size may or may not help to decrease the amount of time spent by hatchlings searching for water. For example, larger hatchling snapping turtles (*Chelydra serpentina*) did not always locate water more quickly than their smaller turtles (Congdon et al., 1999). Although an increased size may not guarantee that the hatchling finds water quickly, the larger size is still beneficial in the environment where small hatchlings become prey for many other species.

In addition to size of the hatchling upon emergence from the nest, there are numerous other factors that contribute to the survivorship of hatchlings. The result of the interaction of these factors throughout nest site selection and embryo development is a

low overall rate of survivorship in hatchling turtles (Pike and Seigel, 2006). For example, only 39.7% of gopher tortoises (*Gopherus polyphemus*) survived from oviposition to hatchling in one study (Pike and Seigel, 2006). Successful populations of turtles are able to overcome the challenges of survival that begin even before the hatchlings emerge into the environment for the first time.

In order for populations to remain successful they must produce viable offspring which are able to survive the current conditions. From nest site selection, to embryo development, and the timing of hatchling emergence there are numerous different strategies employed by turtles in hopes of increasing offspring success. The overall goal of these strategies is to increase the rate of survival of hatchling turtles and provide offspring which can successfully grow and develop and contribute to the existing population. Studying the convergence of the numerous different factors associated with reproductive success is critical in understanding how and why certain populations are successful at various locations. As these locations are affected by development and urbanization, it becomes increasingly important to understand how the factors associated with turtle survivorship are being influenced by human activities. Reproductive ecology is not limited to solely to reproductive events but instead includes influential factors such as habitat use, geographic location, climate, and risk of predation to name a few. These factors may change naturally over time, or they may increase due to encroachment by humans. However, understanding how these factors influence one another and in turn affect reproduction in turtles can help us to understand why reptile populations are declining worldwide (Gibbons et al., 2000) and what conservation measures can be taken

to prevent the downward population trends observed as the earth's human population continues to increase.

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## **PART II. SCIENTIFIC STUDY**

### **The Reproductive Ecology of *Graptemys geographica* in the Central Canal**

**ABSTRACT.**— This study examines the reproductive ecology of a population of common map turtles (*Graptemys geographica*) living in a highly urbanized area of Central Indiana. Radiographs of gravid females (n=10) were used to determine that the average clutch size for *G. geographica* was 13.8 eggs. This clutch size is significantly higher than previous reports of populations in less urbanized areas. Observations of nesting behavior indicate that turtles are easily disturbed and were often forced to relocate during their nesting attempts. While the majority of nesting occurred throughout May and June from 0930 to 1200, observations of attempted nesting occurred much earlier and later than this time period. In addition, some turtles traveled great distances across roads and yards in order to nest.

Although the majority of turtles require aquatic habitats with sufficient resources for daily survival, many turtles also require terrestrial areas suitable for nesting (Marchand and Litvaitis, 2004). Increasingly, these terrestrial habitats are converted from open woodlots and riparian corridors to suburban strip malls and new housing developments through the process of urbanization. The purpose of this study is to examine the reproductive ecology of turtles in an urbanized area with an emphasis on clutch size and timing of oviposition in the common map turtle, *Graptemys geographica*. The common map turtle is a medium-sized aquatic turtle that feeds mostly on a diet of mollusks, and is frequently seen basking in the sun (Minton, 2001). This basking behavior may actually help turtles to ovulate earlier in the season as the thermal warming

of their body helps in allocating more energy towards reproduction (Congdon et al., 2000).

The composition of the turtle assemblage in the Central Canal of Indianapolis, IN was studied in 2002 (Conner et al., 2005) and casual observations have been made for the past fifty years (Minton, 2001); however, none of these studies have focused specifically on the reproductive ecology of *G. geographica* at this location. It is believed that *G. geographica* in the Central Canal travel away from their normal basking sites in order to reproduce (Ryan et al., 2008), but the timing and location of nest sites as well as the clutch sizes are not known for this population. This study hopes to increase our understanding of the reproductive patterns of the common map turtle living within a highly urbanized area in Central Indiana.

In addition to industrialization and agriculture, urbanization is one of the three major threats to freshwater aquatic turtles (Gibbons, 1997). Urbanization may not only inhibit the amount of suitable habitat organisms have to use, but since mobility may be limited it may also result in low genetic diversity within a population (Marchand and Livaitis, 2004). Multiple, interconnected populations are needed for the survival of a species, and fragmentation leads to isolated populations rather than linked populations (Burke et al., 1995). Besides isolating populations from each other, fragmentation may also separate aquatic habitats from suitable terrestrial nesting locations (Marchand and Livaitis, 2004). Increased rates of mortality caused by vehicular collisions have been observed over time with the increase in the construction of roads (Gibbs and Steen, 2005). Due to the fact that female turtles leave their aquatic environment in search of suitable nesting areas, road mortality has affected the female turtle populations

significantly more than the male populations (Gibbs and Steen, 2005). Increased amounts of boating and human alteration of the habitat in and around a northern Indiana lake has resulted in the changes to the composition of aquatic turtle species within that population (Smith et al., 2006). Although boating is not a particular concern for turtle species in the Central Canal, Minton (2001) accurately describes the terrestrial habitat surrounding the Central Canal as a “fatal maze of backyards and busy streets.”

Even though the previous statement appears rather foreboding, not all of the anthropogenic effects associated with urbanization are detrimental for turtles; development helps to increase the amount of open spaces in lawns and backyards which turtles can use for nesting (Marchand and Livaitis, 2004). Despite the pressures associated with urbanization, *G. geographica* appears to be somewhat well adapted to an urbanized environment. Common map turtles have been known to follow large streams into cities and survive in rather urban areas, such as the present Central Canal population (Minton, 2001). The Central Canal not only runs through urbanized areas which are highly disturbed, but the canal itself is far from natural. The Indianapolis Water Company maintains the canal; it promptly removes fallen trees and spends large portions of the summer removing excessive aquatic vegetation that impedes water flow. If left in the canal, this vegetation would provide excellent basking sites for turtles. In Canada, negative population effects have been caused when trees and logs were removed from the turtle’s habitat as part of a river clean-up effort (Chabot et al., 1993).

In the present study, a non-invasive technique of obtaining radiographs was used to determine the number of eggs in an individual’s clutch. This technique, standard practice for over forty years, avoids animal sacrifice and is especially helpful in



examining reproduction in long-lived organisms, such as turtles (Gibbons and Greene, 1979). Avoiding repeated exposure to x-rays helps to minimize the risk for individual turtles (Kuchling, 1998), and information gathered from x-rays can increase the knowledge about an entire population and be used to implement effective conservation methods (Hinton et al., 1997). Calcified eggs are easily identifiable in radiographs (Buhlmann et al., 1995) and the accuracy rate of determining clutch size is often times 100% (Gibbons, 1990). Radiographs are also helpful in determining stages of egg development as increases in calcium deposition are detectable over time (Gibbons and Greene, 1979). Multiple clutching within individuals can also be observed not only by changes in the number of eggs, but also in their position as eggs do not normally experience great changes in position while in utero (Buhlmann et al., 1995).

This study used radiographs to determine average in-utero clutch sizes of the population of *G. geographica* that inhabits the Central Canal. Because similar studies have focused on clutch sizes of populations in more natural areas, the goal of this study is to determine clutch sizes for a population living in a more urbanized environment.

## **METHODS AND MATERIALS**

From mid-May to mid-August of 2007, 0.76m hoop traps were baited with sardines and placed in the Central Canal near the Butler University Campus (Indianapolis, IN, USA) in an attempt to capture turtles inhabiting the area. Previous studies found six species of turtles inhabiting the canal, with the common map turtle, *Graptemys geographica*, being among the most numerous (Conner et al., 2005). The Central Canal was originally intended for transportation uses; however, today it is the

main channel used by the Indianapolis Water Company to transport water to its water treatment facility (Conner et al., 2005). A variety of property types that include parks, residential homes, and commercial businesses and restaurants surround the Central Canal (Ryan et al., 2008).

At any one time three to twelve traps were deployed along the canal from behind the Indianapolis Museum of Art to the intersection of Canal Boulevard and Ripple Road. Traps were checked daily and captured individuals were taken back to the lab for data collection. Each turtle was given a unique mark (Cagle, 1939), and then released at their point of capture following data collection. Sex of the species was determined by secondary sex characteristics. Due to sexual size dimorphism, males are only 20% of the mass of females at maturity (Vogt, 1980). In addition to sex, straight-line carapace length (CL) was measured ( $\pm 1$ mm) with a calipers from the anterior border of the cervical scute to the posterior edge of the two most posterior marginal scutes (Miller and Birchard, 2005). In addition, carapace width (CW) ( $\pm 1$ mm) and mass ( $\pm 1$ g) was measured and recorded. All females were checked for the presence of eggs by palpitation with the index finger. Gravid individuals were then taken to The Avian and Exotic Animal Clinic of Indianapolis where radiography was performed using mammography film (Gibbons and Greene, 1979). Radiographs were performed with the plastron directly atop the mammography film and the carapace closest to the X-ray machine with an exposure time of 0.3 seconds at 50-66 mA (depending on the individual's size).

Carapace length was log-transformed and clutch size data was square root-transformed prior to regression analysis, with a confidence rate of 95%.

In addition to capturing individuals via traps, personal observations were made while biking alongside the canal between the Indianapolis Museum of Art to the south and the intersection of Westfield Boulevard and Guilford Avenue to the north daily throughout June and July of 2007. The timing of this route was varied and included both early morning and evening rides. Turtles seen out of the water or climbing the bank were observed from a distance to determine if the individual was in the middle of a nesting attempt, if digging could be observed, or if a nest containing eggs was present after the turtle's departure.

## RESULTS

### Quantitative

In total, 156 turtles were caught in the traps or by hand (mostly juveniles) throughout the period of data collection. Of these, 85 were *G. geographica*; however, a bias exists as many of these were juvenile 1yr old hatchlings. Only 15 individuals were mature *G. geographica* females with masses greater than 400g. Of these 15 individuals, the first 10 caught between 23 May 2007 and 29 June 2007, were all gravid. The number of eggs possessed by these females ranged from 8-17 with an average of 13.8 eggs per clutch. Overall, clutch sizes of *G. geographica* were significantly larger than all but one of the previous reports conducted in more natural environments (Table 1). No mature females were caught in July and the remaining 5 individuals, caught between 20 August 2007 and 30 August 2007, were not gravid. A significant linear relationship ( $F_{1,8}=9.099$ ,  $P=0.017$ ) was found to exist between carapace length and clutch size (Fig. 1).

Additionally, eight common musk turtles (*Sternotherus odoratus*) were also trapped and taken for radiographs between 23 May 2007 and 19 June 2007. Of the eight caught, seven were gravid with an average clutch size of 6.6 eggs. Clutch size ranged from 4-8 eggs. Carapace length and clutch size in *S. odoratus* were not found to be statistically significant ( $F_{1,5}=2.683$ ,  $P=0.162$ ) (Fig. 2). Compared with reported values for clutch sizes of *S. odoratus* in populations located in more natural habitats, these results indicate that the musk turtles of the Central Canal are producing significantly larger clutches (Table 2).

## Qualitative

### Nesting

One putative *G. geographica* nest was personally observed by a homeowner living at 177 W. Westfield Boulevard. Nesting was said to have taken place on 21 July 2007 at 0645. As the home is on the west side of the canal, the turtle had to cross Westfield Boulevard, come up the driveway of the homeowner and ultimately end up in the mulch below a young tree in the homeowner's backyard. When the mulch was moved away 16 elliptical eggs were found to be inside the nest. Hatchling emergence (19 April 2008) revealed that this nest was actually that of *Trachemys scripta* and not *G. geographica*.

Nesting was also observed in the front yard of a homeowner living at 6120 N Delaware Street (located on the East side of the canal) on 25 May 2007 from approximately 2000 to 2200. Despite excavation of the area, no nest could be located. Another female *G. geographica* was not as fortunate on its nesting foray as it tried to

cross busy Meridian Street at Westfield Boulevard sometime before mid-day and was run over by oncoming traffic. Examination of the carcass found eggs present in the oviducts.

Nesting attempts were observed from 0645 to 2200, with the majority occurring between 0930 and 1200 from the middle of May through the end of June. Simply passing within view of a *G. geographica*, whether basking in the sun or up on the bank, almost always caused a sudden retreat back into the water. The skittish reaction of the turtle is surprising due to the large number of pedestrians, bikers, and even motorists that often come within 1-5m of individuals. Many of the aborted nesting attempts that I witnessed were likely terminated by my presence. Many times I would not even notice a turtle out of the water and up on the bank until I was almost directly above the individual. By that time it was too late, and the turtle would slide back down the bank and disappear into the water. However, one aborted nesting attempt I observed from a distance may have been due to excessively dry soil, which may make digging a nest almost impossible. The unseasonably hot and dry summer of 2007 could have prevented females from being able to dig a sufficiently deep nest. Increased nesting abortions have been noted in Blanding's turtles (*Emydoidea blandingii*) under similar conditions (Congdon et al., 2000). Also, the banks of the canal have been compacted over the years resulting in soil that is difficult to dig in even when using a metal trowel.

The several nesting attempts I observed all involved females digging with their hind legs while their head faced away from the water. After the female had left the scene, close examination of the soil on multiple occasions showed that the ground was wet as if water had been voided from the cloacal bladder in order to soften the ground (Cagle,

1950). Despite the release of water in order to soften the ground, for some reason the nesting locations that I observed must not have been suitable for other unknown factors.

## Predation

I found evidence of nest predation on four separate occasions. Depredated nests were identified by the presence of eggshell fragments near the nest location. On one occasion, the nest was initially observed being dug and five days later it was depredated before excavation could determine the number of eggs contained within. This is not surprising as predation for Bladning's turtles was found to be the highest after the first full day of nest construction (Congdon et al., 2000), while no predation of *Chrysemys picta* nests occurred after twelve days (Rowe et al., 2005). On one occasion, the remaining egg shells had visible claw marks on them suggesting that the predator was a mammal. Most likely this predator was a raccoon given the urban setting. This same depredation event resulted in egg shell fragments located around the nest but also remnants of shells approximately 4m away in the lawn of a homeowner. This circumstantial evidence suggests that the predator transported the eggs away from the canal's steep bank and into the flat and somewhat protected area of a lawn in order to consume them.

## DISCUSSION

Low capture rates throughout the summer (in comparison with previous years at similar locations) could have been caused by the fact that the turtles have learned to avoid the traps (Souza and Abe, 2001). Trapping has been occurring regularly since 2001 in the

Central Canal (T. Ryan, personal communication). Even when traps were re-baited weekly and re-positioned in order to cover a greater area, traps were more successful in providing basking sites for turtles than they were in eliciting turtles to actually enter the traps.

The low number of nests located may be an indicator of how the population of *G. geographica* has managed to remain successful despite the increased urbanization occurring in its surrounding habitat. Even vigilant efforts to observe and find nests were only minimally successful. Aside from the few nests and nesting attempts I actually observed, it appears that radio telemetry would probably be necessary to track the nesting behavior of these easily startled organisms.

Urbanization appeared to affect the nesting behavior of *G. geographica* in the Central Canal. A previous study (E. Shoening and T. Ryan, unpublished) showed that most nesting by *G. geographica* occurs in residential and commercial areas, specifically in landscaped flower/shrub beds. Due to the fact that these turtles are easily distressed by disturbances along the canal, they must devote increased amounts of energy to locating a suitable nesting location. Each aborted nesting attempt costs energy when the female must relocate her nest after being scared away from her original nest site. Also, nesting forays which cause the turtle to cross busy streets increase the chances of mortality compared with populations living in more natural and/or protected areas.

### **Clutch size**

Previous studies suggest that the average clutch size for the twelve species of *Graptemys* ranges from 4.7-14.1 eggs (Lindeman, 2005). The average clutch size for

*Graptemys geographica* in this study (n=10) was 13.8 eggs with a range of 8-17.

Although this falls within the range of the genus, it is a bit different than other reports for the species. Only one other report of clutch sizes as high as 19 has been reported in Wisconsin by Vogt (1980). The study area of this report was a fairly natural area of a pool of the Mississippi River (Vogt, 1980). Minton (2001) stated that the clutch size range for *G. geographica* is between 10 and 16, and Carr (1952) reported that there are 10-16 eggs per clutch in *G. geographica*. The clutch size of *G. geographica* in the Central Canal was significantly higher than three other studies all of which occurred in more natural settings (Table 1). In addition to overall larger clutches, female *G. geographica* with larger body sizes are producing clutches containing a greater number of eggs compared to smaller females (Fig. 1). This suggests that larger females have more energy to allocate to reproduction in addition to having larger body cavities to retain the eggs.

Larger clutch sizes were also found in *S. odoratus* when compared to previous reports (Table 2). This discrepancy may be caused by the fact that ten out of the eleven clutch sizes were determined via radiography and not by counting eggs actually laid in nests. When Condgon et al. (2000) compared clutch size from radiographs with that of nest clutch size they observed two fewer eggs in the nest of Blanding's turtles. These researchers suggested that this discrepancy may be due to predation by burrowing mammals. Another possibility is that increased rates of predation in an urban setting may select for individuals that lay larger clutches in hopes of increasing offspring survival. Although a statistical relationship between clutch size and carapace length in *S. odoratus* could not be established, this is most likely due to the small sample size (n=7). Within



this cohort, carapace length is responsible for over 32% of the variance observed in clutch size indicating the likely significance if the analyzed sample size had been larger.

## **Predation**

Although predation on eggs seemed somewhat high, this is not uncommon in turtles. Predation appears to be a significant factor for turtles of numerous species. Butler et al. (2004) noted that raccoons were the main nest predator of Carolina diamondback terrapins (*Malaclemys terrapin centrata*) on an island in Florida. Separate studies found raccoons to be the primary nest predator of Midland painted turtles (*Chrysemys picta marginata*) (Rowe et al., 2005) and Blanding's turtles (*Emydoidea blandingii*) (Congdon et al., 2000). A long-term study of Blanding's turtles documented a predation rate of 100% nine out of ten years (Congdon et al., 2000). Over a period of twenty-three years, the same study reported a predation rate of 78.2%. This same study also found that rates of predation decreased over time after the nest was laid; this suggests that predators may use olfactory clues that gradually fade away over time in order to locate nests (Congdon et al., 2000). In addition, the fluid released from the cloacal bladder may help soften the soil and facilitate digging the nest, but it may also inadvertently attract predators (Cagle, 1950). Predation affects hatchlings and juveniles to a greater extent, but increased mortality of mature individuals actually has much greater ecological consequences for a population than egg predation and predation rates on hatchlings and juveniles (Congdon et al., 2000).

## Implications

When data collected from individuals can be applied to a population, determining fecundity for an entire population is possible. Estimates of population size make it possible to extrapolate this information and estimate total egg production of a population if the number of mature females throughout the population can also be determined (Litzgus and Mousseau, 2003). In populations, this also means factoring in clutch frequency. Although lack of recaptured individuals prevented clutch frequency from being determined in this study, studies that have examined the enlarged follicles and corpora lutea of *G. geographica* estimate that two or even three clutches may be produced per season in some populations (White and Moll, 1991).

Overall, long-term monitoring is essential in determining if (and how) humans may be responsible for altering the composition and abundance of species within a population (Smith et al., 2006). Long-term studies will aid tremendously in making conservation and management decisions for threatened populations; as without the existence of previous long-term studies many conservation practices are implemented in times of crisis without sufficient knowledge of how a population may have been impacted and what can be done to help it recover (Congdon et al., 2000). Moreover, rather sudden changes to the turtles' habitat necessitate long-term studies as turtles usually exhibit delayed responses to environmental changes (Marchand and Livaitis, 2004). Understanding the life history characteristics, such as reproduction, will become increasingly more important as the world reptile population continues to decline (Gibbons et al., 2000, Klemens, 2000 and Bager et al., 2007).

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## TABLES

Study	Location	Mean	Range	t value when df=9	Probability
Nagel et al., 2004	Central Pennsylvania	10.6	6-15	<b>t=3.244</b>	P=0.005
Baker et al., 2003	Fulton Co., Indiana	7.3	3-13	<b>t=6.588</b>	P=0.00005
White and Moll, 1991	Dallas Co., Missouri	10.1	6-15	<b>t=3.750</b>	P=0.0023
Vogt, 1980	Wisconsin	13.6	8-19	t=0.203	P=0.4218

**Table 1.** Previous reports of clutch size in *G. geographica*. The t value was computed based on a one sample t-test comparing these studies to our sample clutch size of 13.8 (n=10). **BOLD** denotes significant differences.

Study	Location	Mean	Range	t value when df=6	Probability
Mitchell, 1988	Virginia	2.7	1-5	<b>t=7.385</b>	P=0.0002
Ernst, 1986	Lancaster County, Pennsylvania	3.25	2-4	<b>t=6.343</b>	P=0.0004
Iverson, 1977	Northern Florida	3.2	2-6	<b>t=6.438</b>	P=0.0003
Tinkle, 1961	Northern States (including Indiana)	4.6	2-7	<b>t=3.787</b>	P=0.0046
Tinkle, 1961	Southern States	2.2	1-4	<b>t=8.331</b>	P=0.00008
Gibbons, 1970	Welaka, Florida	2.4	1-4	<b>t=7.953</b>	P=0.0001
McPherson and Marion, 1981	Central Alabama	2.6	1-6	<b>t=7.574</b>	P=0.0001

**Table 2.** Previous reports of clutch size in *S. odoratus*. The t value was computed based on a one sample t-test comparing these studies to our sample clutch size of 6.6 (n=7). **BOLD** denotes significant differences.

## FIGURES

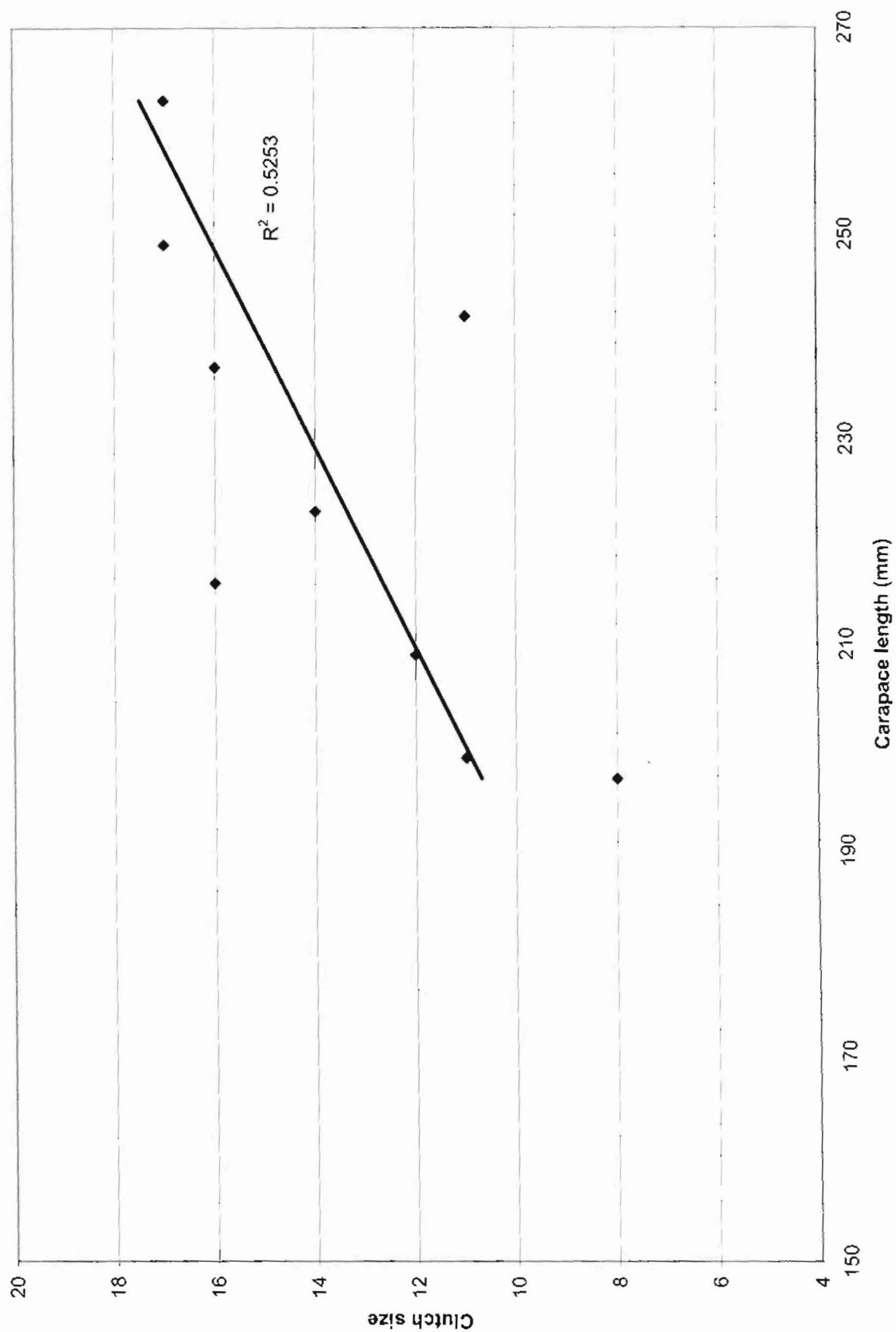


Figure 1. The effect of body size on clutch size in *G. geographica*.

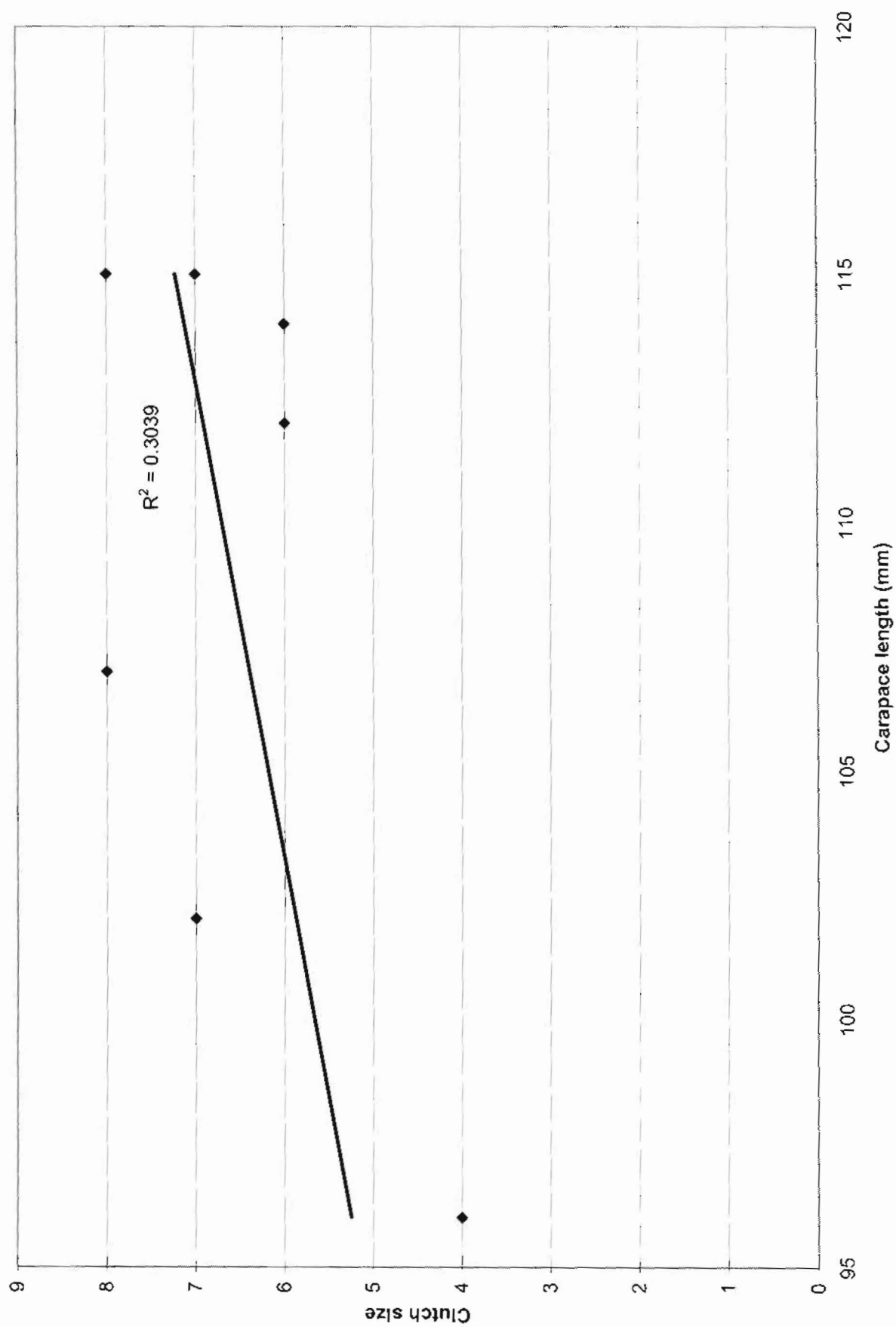


Figure 2. The effect of body size on clutch size in *S. odoratus*.